recording actually provides a means to "separate" the various permutations of each combination of heteromers. According to these data, combinatorial assembly can provide pores with characteristic responses over a wide range of analyte concentrations.

Other embodiments are within the following claims.

What is claimed is:

1. A mutant staphylococcal alpha hemolysin polypeptide comprising a heterologous amino acid, wherein said heterologous amino acid binds an analyte and wherein said polypeptide assembles into a heteroheptameric pore assembly in the presence of a plurality of wild type staphylococcal alpha hemolysin polypeptides.

2. The polypeptide of claim 1, wherein said heterologous amino acid occupies a position in a transmembrane channel of said heptameric pore assembly.

3. The polypeptide of claim 2, wherein said heterologous amino acid projects into the lumen of said transmembrane channel.

4. The polypeptide of claim 2, wherein said heterologous amino acid occupies a position in a stem domain of said polypeptide.

5. A staphylococcal alpha hemolysin (αHL) polypeptide comprising at least two non-consecutive heterologous amino acids in a stem domain of said polypeptide, wherein each of said heterologous amino acids binds a metal.

6. The polypeptide of claim 5, wherein said amino acids occupy two or more of the following positions of SEQ ID NO:1: 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147 or 149.

7. The polypeptide of claim 5, wherein said amino acids occupy two or more of the following positions of SEQ ID NO:1: 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148.

8. The polypeptide of claim 5, wherein said polypeptide comprises at least three non-consecutive heterologous amino acids in the stem domain of said polypeptide.

9. The polypeptide of claim 5, wherein said polypeptide comprises at least 4 non-consecutive heterologous amino acids in the stem domain of said polypeptide.

10. The polypeptide of claim 9, wherein said amino acids occupy positions 123, 125, 133, and 135 of SEQ ID NO:1.

11. The polypeptide of claim 10, wherein said polypeptide is 4H.

12. The polypeptide of claim 1, wherein said amino acid is selected from the group consisting of Ser Thr, Met, Trp, and Tyr.

13. The polypeptide of claim 12, wherein said amino acid is selected from the group consisting of Glu, Asp, Cys, His.

14. The polypeptide of claim 13, wherein said amino acid is His.

15. A staphylococcal alpha hemolysin (αHL) polypeptide comprising at least two non-consecutive heterologous amino acids in a stem domain of said polypeptide, wherein each of said heterologous amino acids binds an organic molecule.

16. The polypeptide of claim 15, wherein said organic molecule is an explosive.

17. The polypeptide of claim 15, wherein said amino acids occupy two or more of the following positions of SEQ ID NO:1: 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147 or 149.

18. The polypeptide of claim 16, wherein said polypeptide is 123W/125W.

19. The polypeptide of claim 1, wherein said polypeptide further comprises a second heterologous amino acid at a site distant from said stem domain.

20. The polypeptide of claim 19, wherein said second heterologous amino acid is a Cys residue at position 292 of SEQ ID NO:1.

21. A heptomeric pore assembly comprising a mutated staphylococcal αHL polypeptide (MUT), wherein said MUT is an analyte-binding αHL polypeptide.

22. The pore assembly of claim 21, wherein said pore assembly is a heptamer having the formula $WT_{7-n}MUT_n$, wherein n is greater than zero and less than seven.

23. The pore assembly of claim 21, wherein said analyte-binding αHL polypeptide comprises a heterologous amino acid at a position in a transmembrane channel of said pore assembly, wherein said heterologous amino acid binds a metal.

24. The pore assembly of claim 21, wherein said pore assembly is a heptamer having the formula $WT_{7-n}M_n$, wherein n is greater than zero and less than seven.

25. The pore assembly of claim 17, wherein said analyte-binding αHL polypeptide is 4H.

26. The pore assembly of claim 21, wherein said analyte-binding αHL polypeptide is 123W/125W.

27. The pore assembly of claim 25, wherein the pore assembly is a heptamer having the formula $WT_{7-n}4H_n$.

28. The pore assembly of claim 27, wherein the pore assembly is a heteroheptamer having the formula $WT_6 4H_1$.

29. A digital biosensor device comprising the pore assembly of claim 21.

30. The device of claim 29, wherein said analyte-binding αHL polypeptide comprises at least two non-consecutive heterologous amino acids in the stem domain, wherein each of said heterologous amino acids binds a metal.

31. The device of claim 29, wherein said analyte-binding αHL polypeptide comprises a chelating molecule in the stem domain of said polypeptide.

32. The device of claim 29, wherein said device detects binding of a metal ion to said analyte-binding αHL polypeptide.

33. The device of claim 32, wherein said device detects a single channel current.

34. The device of claim 32, wherein said device detects a current through two or more channels.

35. A method of detecting the presence of an analyte in a test sample, comprising

(a) contacting said sample with the pore assembly of claim 21; and

(b) detecting an electrical current in a digital mode through two or more channels, wherein a modulation in current compared to a current measurement in a control sample lacking said analyte indicates the presence of said analyte in said test sample.

36. A method of detecting the presence of an analyte in a test sample, comprising

(a) contacting said sample with the pore assembly of claim 21;

(b) detecting an electrical current in a digital mode through a single channel, wherein a modulation in current compared to a current measurement in a control sample lacking said analyte indicates the presence of said analyte in said test sample.

37. The method of claim 36, wherein said analyte is a metal ion.

38. The method of claim 37, wherein said metal ion is Zn(II).

39. The method of claim 37, wherein said metal ion is Co(II), Cu(II), Ni(II), or Cd(II).

40. A method of identifying an unknown analyte in a mixture of analytes comprising,

(a) contacting said mixture with the pore assembly of claim 21;

(b) detecting an electrical current in a digital mode through two or more channels to determine a mixture current signature;

(c) comparing said mixture current signature to a standard current signature of a known analyte, wherein a concurrence of said mixture current signature and said standard current signature indicates the identity of said unknown analyte in said mixture.

41. The method of claim 40, wherein each of said known and unknown analytes is a metal ion.

42. A method of identifying an analyte in a mixture of analytes comprising,

(a) contacting said mixture with the pore assembly of claim 21;

(b) detecting a single channel current in a digital mode to determine a mixture current signature;

(c) comparing said mixture current signature to a standard current signature of a known analyte, wherein a concurrence of said mixture current signature and said standard current signature indicates the identity of said unknown analyte in said mixture.

43. The method of claim 42, wherein each of said unknown and known analytes is a metal ion.

44. The method of claim 43, wherein said metal ion is Zn(II).

45. The method of claim 43, wherein said metal ion is Co(II), Cu(II), Ni(II), or Cd(II).